Identification and Antibiotic Susceptibility of Salmonella Isolated from Diarrhoeal Children of Displaced People: Dar Elsalam, Khartoum, Sudan

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Dedication

I would like to dedicate this humble piece of effort to those who have waited so long to see it a reality, after appreciable support and encouragement.

To my father Nyichar and mother Bukodho, brother Chol and sisters

To my wife, Regina and to my lovely kids, Nyichar, Acwanyo, Lam, Mer and Wodo

With great love and gratitude

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Abstract

In this study, Salmonella associated with infant diarrhoea was studied in a group of 50 children from people who were brought to Dar-assalam camp at the vicinity of Omdurman Province, Khartoum State. Diagnostic procedures (cultural, biochemical and serological) were carried out to optimize detection of Salmonella species in the samples collected (50 stool samples and 20 serum samples). Serum samples were collected from the cases found positive in isolation. Salmonella was isolated from 20 stool samples (40%). Nine cases were positive for S. Typhi, four were positive for S. Paratyphi and seven were positive for S. Typhimurium. Isolation results of S. Typhi and S. Paratyphi were confirmed by serology (Widal test), where sera of cases positive for S. Typhimurium isolation, were negative (insignificant antibody titre). Co-infections were also detected. Most of the primary cultures of the stool samples were mixed cultures. Escherichia coli was isolated from ten and Shigella from four. Also, in the direct wet mounts of stool samples, Amoeba was observed in six, flagellates in five and worms (Scaris and *Hymenolepis nana*) in two samples.

The antibiotic susceptibility test was performed for the 20 Salmonella isolates against eleven antimicrobial agents, namely, ciprofloxacin, cefotaxime, chloramphenicol, kanamycin, gentamycin, ampicillin, streptomycin, nalidixic acid, cephalexin, tetracycline and Septrin® (sulphamethoxazole and trimethoprim). All the isolates were highly sensitive to ciprofloxacin, which was the most potent antibiotic used, followed by cefotaxime. All *S.* Typhimurium isolates were resistant to chloramphenicol, while *S.* Typhi and *S.* Paratyphi isolates showed high to moderate sensitivity

to chloramphenicol. All the isolates of the three species were moderately sensitive to Septrin. On the other hand, all the isolates were resistant to other antibiotics. The results of the antibiotic susceptibility showed that the isolates were sensitive only to a few antibiotics and this may indicate emerging resistance to commonly used antibiotics. Therefore, in routine laboratory diagnostic work, beside pathogen isolation and identification, control measures should also include drug-sensitivity test.

ملخص الأطروحة

أجريت هذه الدراسة لمعرفة علاقة السلمونيلة بإسهالات أطفال النازحين بمعسكر دار السلام بأطراف محافظة أمدرمان بولاية الخرطوم. طرق التشخيص التي تم استخدامها للتعرف على أنواع السلمونيلة شملت الخصائص المزرعية، البيوكيموحياتية والمصلية وذلك فى خمسين (50) عينة برازية وعشرين (20) عينة مصل. عينات المصل تم أخذها فقط من الحالات التي تم عزل السلمونيلة من العينات البرزاية لها. تم عزل السلمونيلة من عشرين من العينات البرازية وذلك بنسبة 40%. تم تعريف تسعة من العزلات على أنها سلمونيلة تيفية، أربعة على أنها سلمونيلة نظيرة التيفية وسبعة على أنها سلمونيلة تيفية فأرية. عزلات السلمونيلة التيفية والسلمونيلة نظيرة التيفية تم تأكيدها بواسطة إختبار فيدال المصلي؛ بينما الأمصال التي تم أخذها من الحالات التي عزلت منها السلمونيلة التيفية الفأرية أظهرت نتيجة سالبة بواسطة هذا الاختبار.

بعد زراعة العينات البرازية والفحص المباشر لها بالميكروسكوب تبين أن معظم العينات تحتوى على أكثر من نوع واحد من البكتريا كما أن بعضها يحتوى على بعض أنواع الطفيليات. تم عزل الأشريكية القولونية من عشرة من هذه العينات والشيغلة من أربعة. كذلك في المسحات اللينة تمت ملاحظة تواجد الأميبة في ستة من العينات، السوطيات فى خمسة والديدان (الصفر والمحرشفة القزمة) في اثنين.

تم إجراء إختبار الحساسية للمضادات الحيوية لكل عزلات السلمونيلة (20 عزلة) وذلك لأحدى عشر مضاد حيوي: سبروفلوكساسين، سيفوتاكسيم، كلورامفينيكول، كاناميسين، جنتاميسين، أمبسلين، استربتوميسين، ناليديكسك اسيد، سيفالكسين ، تتراسايكلين وسبترين. كل العزلات أظهرت حساسية عالية جداً للسيبروفلوكساسين

والذى تلاه سيفوتاكسيم. كل عزلات السلمونيلة التيفية الفأرية أظهرت مقاومة للكلورامفينيكول بينما عزلات السلمونيلة التيفية ونظيرة التيفية أظهرت حساسية عالية إلى متوسطة. كل عزلات أنواع السلمونيلة الثلاثة التي تم عزلها أظهرت حساسية متوسطة للسبترين. فى الجانب الآخر، كل العزلات أظهرت مقاومة لبقية المضادات الحيوية.

نتيجة اختبار حساسية عزلات السلمونيلة للمضادات الحيوية أبانت أن العزلات كانت حساسة لعدد قليل من المضادات الحيوية وهذا ربما أشار إلى ظهور مقاومة للمضادات الحيوية المستخدمة عادة. ولذلك عند التشخيص المعتاد بالمعامل بجانب عزل البكتريا والتعرف عليها، يجب كذلك إجراء اختبار الحساسية للمضادات الحيوية لتعزيز طرق المكافحة.

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1. INTRODUCTION

Salmonellosis is a bacterial illness characterized by acute abdominal pain, diarrhoea, and often fever that begins 12 hours to five days after infection (Jawetz *et al.*, 1966). Dehydration and electrolyte imbalance are the important consequence of the diarrhoea due to Salmonella infections. In cases of enterocolitis, faecal excretion of the organism usually persists for several days or weeks beyond the acute phase of illness. Duration of excretion of the organism may be increased by the misuse of antibiotics (Buchwald and Blaser, 1984).

The incidence of infection is highest in infants, elderly and young children (Wilson and Miles, 1964). The majority of human infections are thought to result from ingestion of focally contaminated food (food-borne disease) or water. Undercooked or raw products of animal origin such as eggs, milk, meat, fish, and poultry have been implicated as common sources of human salmonellosis (Jay, 2000). A wide range of domestic and wild animals are carriers of *Salmonella*, including poultry, pigs, cattle, dogs, cats, rodents, iguanas, tortoises, turtles, terrapins. Though uncommon, person-to-person spread can occur from diseased people, convalescent carriers and mild and unrecognized cases (Buchwald and Blaser, 1984).

Salmonellosis is one of the diarrhoeal diseases that might cause illness in developing countries. They contribute to the deaths of 4.6 - 6 million children annually in Asia, Africa, and America (Schelotto *et al.*, 1991). However, the morbidity is equally important in poor countries, and in

tropical climates where it has been estimated that each child suffers up to 15 to 19 episodes of diarrhoea per year (Schelotto *et al.*, 1991).

In past decades, diarrhoea and malnutrition (closely related pathologies) has contributed significantly to infant mortality, as it reached a figure of 120 fatalities per 1,000 new born children (Schelotto *et al.*, 1991). Accompanying general improvement of the quality of live, the World Health Organization (WHO) instituted a number of local programmes of diarrhoeal disease control. This has included promotion of breast-feeding, oral rehydration therapy, and specific health education (Schelotto, *et al.*, 1991). Concequently, a gradual decrease in the prevalence of these diseases was registered especially after the year 1980 (Schelotto *et al.*, 1991); with the resultant decline in global infant death rate. Morbidity and mortality of children diarrhoea are currently associated with cases that are attributed to improper feeding or dehydration, which may lead to extra-intestinal or systemic involvement. This in addition to persistent diarrhoea that occurs especially in infants from low socio-economic groups. Hence displaced people are mostly vulnerable to the real diseases. It is an undisputable fact that the Sudan is one of the countries that suffer from high infant death rate. This high mortality rate amongst Sudanese children is mainly a result of the prevalence of diarrhoeal diseases (Erwa, 1966). The poor standard of sanitation and hygiene makes diarrhoeal diseases the most frequent and the main causes of deaths in children compared with other diseases. In the Sudan, the number of deaths due to diarrhoeal diseases was 10,816 in 1970 (Erwa, 1975).

The objectives of this study were to:

1. perform a primary investigation of Salmonella associated with diarrhoea in children of displaced people of Dar-assalam camp.
2. to isolate the bacteria from faecal samples and to the serotype level by cultural, biochemical and serological methods.
3. to study the susceptibility of the isolates to some locally available antibiotics.
4. to draw recommendations based on the obtained results aimed at treatment and control of Salmonella infection.

2. LITERATURE REVIEW

2.1 The genus Salmonella

The genus S*almonella* spp is named after an American veterinary pathologist, Daniel E. Salmon, who first isolated the organism in 1884 from porcine intestine (Ryan, 2004). Salmonella spp cause salmonellosis in both man and animals. Human salmonellosis is characterized by an acute abdominal pain, diarrhea; and often fever that begin 12 hours and extend to five days after infection in cases of gastroenteritis (Jawetz *et al*., 1966).

2.1.1 Normal habitat.

Salmonellae are common inhabitants of human and animal intestines. Also, it is found in insects, soil and water (Cheesbrough, 1984).

2.1.2 Antigenic structure of salmonellae

Salmonella has a somatic (O) antigen which is a component of the cell wall of the organism and is a lipopolysaccharide and group specific. The flagellar (H) antigen, which is situated in the flagella, is a protein and species specific. The Vi (virulence factor) antigen, which is associated with bacterial capsule (K envelope), is a polysaccharide which forms an envelope surrounding the surface of the organism protecting the O antigen from bactericidal agents. It is related to invasiveness and the effectiveness of vaccines and can make detection of O antigen difficult. *Salmonella typhi* produces an endotoxin which forms the outer portion of the cell wall and it is the released O antigen, which is composed of a lipo-polysaccharide and lipid A which contribute to the pathogenesis of typhoid fever (Manson-Bahr *et al.,* 1978; Brooks *et al*., 1998)

2.1.3 Classification of Salmonella

Salmonellae can be classified according to biochemical, serological and genetical analysis.

2.1.3.1 Biochemical classification

The Salmonellae contain a single genus, Salmonella, are named other the American microbiologist, David E.Salmon. On the basis biochemical reactions, Kauffmann proposed that Salmonella can be classified into six subgenera (Paniker and Vilma, 1997).

- Subgenus 1: It is the largest and medically the most important group that contains all the species commonly causing human and animal infections. It includes most of the serotypes, *S. Typhi, S. Choleraesuis, S. Paratyphi* and *S.Galinarum.*
- Subgenus 2: This subgenus contains mostly species isolated from reptiles, for examples *S. Salmae.*
- Subgenus 3: This contains bacilli, formerly designated as (Arizona), originally isolated from lizards but subsequently found in reptiles, birds, domestic animals and humanbeings many of them are prompt lactose fermenters and are subdivided into: Salmonella subgroup 3a: S. Arizona. Salmonella subgroup 3b: S. diarzone
- Subgenus 4: Those strains are rarely encountered and may be considered as atypical members of subgenus 2, e.g. S.houtenae.
- Subgenus 5: An example of this subgenus is S.bongori.
- Subgenus 6: An example of this subgenus is S.choleraesuis subspecies indic (Paniker and Vilma, 1997).

2.1.3.2 Serological classification (Kauffmann White classification)

Salmonella subcommittee (1934), proposed that serology was the ultimate criterion in the classification of Salmonella (Barrow and feltham, 1993). Kauffmann-White scheme for classification was first developed in 1934 and it classifies salmonellae in to different O groups or O serotypes, each of which contains a number of serotypes possessing a common O antigen not found in other O groups. The O groups, first defined, were designated by capital letters A to Z and those discovered later by the number (51- 67) of the characteristic O antigen (David and Mass, 1989). According to this scheme, salmonellae are classified into groups (Table I). The identification of serotypes is based on the detection of O and H antigens (phase I and II) in the unknown serotype, which is indicated by the agglutination of these antigens with the homologous antibodies in the test sera (Cheesbrough, 1984). This scheme has been modified by Brenner (1998). To incorporate only three species with in the genus *Salmonella Typhi*, *S. Choleraesuis* and *S. Enteritidis* and all formal species are serovars these are *S. Enteritidis.*

2.3 Classification by Deoxyribonucleic Acid (DNA) hybridization:

Modern taxonomical techniques, especially DNA studies, have shown that all the members of the genus Salmonella and the former genus Arizona are so closely related that they should all be considered as belonging to a single species, in a genetic, phylogenetic and evolutionary sense (David and Mass, 1989).

DNA hybridization studies have shown all salmonellae to be genetically identical (cheesbrough, 2000). A new species named *Salmonella enterica* has been coined to include all salmonellae. *S. enterica* is classified into seven subspecies based on DNA reassociation tests (Ananthanarayan

Paniker, 1997), each with its own phenotypic characteristic and history (Cheesbrough, 2000).

These subspecies are named enterica, salamae, arizona, diarizonae, houtenae, longori and indica. Subspecies enterica corresponds to the former subgenus I (Paniker and Vilma, 1997).

This genetically based new classification system has not yet been widely adopted by medical microbiologist (Cheesbrough, 2000). Deoxyribonucleic Acid (DNA) classification and nomenclature, while being taxonomically correct, would be too complicated for use in clinical bacteriology. For example, the taxonomically correct name for typhoid bacillus would be (*Salmonella enterica*, subspecies enterica, serotype typhi). Therefore, the old practice of referring to clinically important Salmonella serotypes by the species name continues in clinical bacteriology. Important pathogens such as *S. Typhi, S. Paratyphi* A and B can father be typed for epidemiological purposes by phasge susceptibility, biochemical properties, and bacteriocin production (Stratton *et al*., 2001). Recently, all strains in the genus are assigned to only two species, *S. Bongori* and *S. enterica*.

Disease-causing salmonellae have been re-classified into Salmonella *enterica*, which has numerous strains or serovars belong to six subspecies. Isolates from humans and warm-blooded animals belong to *S. enterica* subspecies *enterica*. The vast majority of human isolates (> 99.5 %) are subspecies of *S. enterica*. For simplicity, (Centres for Disease Control and Prevention, Atlanta, USA) recommended that Salmonella spp. be referred to only by their genus and serovar; e.g. *Salmonella typhi* instead of *Salmonella*

enterica subspecies *enterica* serovar *typhi*. Now the genus Salmonella may include more than 2500 different serotypes that differ in their host range and ability to cause disease despite their close genetic relatedness (Mansfield and Forsythe, 2000, Mortimer *et al*., 2004).

S. typhi can usually be distinguished from other serotypes by considerable overlap in antigenic composition which is responsible for the cross-reactivity, which is commonly encountered in typhoid fever (Guerrant, 1987).

Table I. Kauffmann–White classification of salmonellae

Salmonella spp. group	O antigens	H antigens	
Group A		Phase I	Phase II
S. paratyphi A	1, 2, 12	a	-
Group B			
S. paratyphi B	1, 4, 5, 12	b	1, 2
S. derby	1, 4, 5, 12	fg	(1, 2)*
S. typhimurium	1, 4, 5, 12	i	1, 2
S. heidelberg	(1), 4, 5, (12)	r	1, 2
Group C			
S. choleraesuis	6, 7	c	1, 5
S. paratyphi C	6, 7, (vi)	c	1, 5
S. oranienburg	6, 7	m, t	-
S. garoli	6, 7	i	1, 6
S. thomson	6, 7	k	1, 5
S. bareilly	6, 7	y	1, 5
Group D			
S. typhi	9, 12, (vi)	d	-
S. enteritidis	1, 9, 12	(g, m)	-
S. gallinanum	1, 9, 12	-	-
Group E1			
S. welterveden	3, 10	r	z_6
S. anatum	3, 10	e, h	1, w
Group G2			
S. durham	13, 23	6	e, n, z_6
S. worthington	1, 13, 23	z_{29}	-

S. cubana		b, e	-

* Brackets indicate that the antigen may be present or absent

2.1.4 Bacteriological characteristics of Salmonella

In most aspects, the salmonellae are quite similar to other enteric bacteria in morphology, colonial characteristics, and some similarities in biochemical activities. They are Gram negative, nonsporing, actively motile (except *S. gallinarum*), rod-shaped or coccobacillary organisms, which are seldom larger than 3 µ in length by 0.6 µ in width; long filamentous forms are occasionally obtained from infections of the urinary tract or when the organisms are grown in liquid medium. Encapsulated forms occur when the salmonellae are incubated at 20 ºC or less; although optimal growth occurs at 37 ºC. Such encapsulated types form mucoid colonies. All the salmonellae grow well on ordinary culture media, forming colonies similar to those of the coliform group (Barrow and Feltham, 1993; Cheesbrough, 2000).

Biochemical characteristics are important means of differentiating salmonellae from species of other genera of the enteric group. For example, the salmonellae can be differentiated from the coliform group by their inability to ferment lactose. In general, the inability of the salmonellae to ferment lactose, sucrose, or salicin and to produce indole and gelatinase are their most reliable generic biochemical characteristics; other fermentation reactions vary considerably. Also, biochemical characteristics aid in differentiation of Salmonella species. *S. Typhi* can be differentiated from *S.Paratyphi* by differences in fermentation of carbohydrates in addition to other biochemical reactions (Krieg and Holt, 1984).

The salmonellae, like other nonsporing bacteria, are not particularly resistant

to physical and chemical agents. They are usually killed within 15 minutes at 60 ºC. On the other hand, they can survive for months in soil during the winter and for several days in cool contaminated water. They are resistant to dyes and inhibitory substances such as brilliant green, sodium deoxycholate, and selenium compoundsin contrast. Citrate and sodium tetrathionate can even enhance their growth. When such substances are incorporated in culture media, coliform bacilli are inhibited while salmonellae can be separated from them (Burrnett *et al.*, 1962).

2.2 Salmonella infections in man

2.2.1 Human Salmonella spp.

Salmonella species that cause human salmonellosis are *S. typhi*, *S. paratyphi* (*A, B, C*), *S. enteritidis*, *S. typhimurium*, *S. enteritidis heidelberg*, *S. enteritidis newport*, *S. hadar*, *S. enteritidis agona*, *S. enteritidis montevideo*, *S. oranienburg*, *S. muenchen*, *S. enteritidis Thompson* and *S. infantis*.

Salmonellosis includes several syndromes (gastroenteritis, septicemia, focal infection, and enteric fever (typhoid) which is the most important Salmonella infection in humans. It is an acute systemic disease resulting from infection with *Salmonella typhi*, which affects only humans. However, a mild enteric fever, similar to typhoid, can be caused by other Salmonella serotypes and is termed paratyphoid fever (Samuel, 1996).

2.2.2 Incidence and epidemiology

The incidence of infection is highest in infants, elderly and young children due to immune response (Wilson and Miles, 1964). Salmonellosis is one of the diarrhoeal diseases that are frequently reported in developing countries. They contributed to the deaths of 4.6 - 6 million children annually in Asia,

Africa and America. Morbidity is also especially important in poor countries and tropical climates which have been estimated that each child suffers up to 15 to 19 episodes of diarrhoea per year (Schelotto *et al.*, 1991). According to the findings of Mohamed (1999), the most prevailing Salmonella species in the Sudan is *S. typhi*.

In past decades, diarrhoea and malnutrition contributed significantly to infant mortality, which reached a figure of 120 per 1,000 new born children (Schelotto *et al.*, 1991). Accompanying general improvement of the quality of live, the World Health Organiozation (WHO) instituted a number of local programmes of diarrhoeal disease control, including promotion of breast-feeding, oral dehydration, therapy and specific health education (Schelotto *et al.*, 1991). The incidence of salmonellosis has steadily decreased. The decrease in incidence has been coincident with the improvement in socio-economic conditions and is specifically related to development of pure water supplies, effective sewage disposal and pasteurization of milk. Salmonellosis continues to occur on large scale in countries where sanitation is suboptimal. Morbidity and mortality of children are currently associated with cases that evolve without improper feeding or dehydration, which cause extra-intestinal or systemic involvement, or persistent diarrhoea that occur especially in infants from low socio-economic groups. Displaced people are the most affected by diarrhoeal diseases. It is an undisputable fact that the Sudan is one of the countries that suffer from high infant death rate. This high mortality rate amongst Sudanese children is mainly a result of the prevalence of diarrhoeal diseases (Erwa, 1966). The poor standard of sanitation and hygiene makes diarrhoeal diseases the most frequent and the main causes of deaths in children compared with other diseases. The number

of diarrhoeal deaths due to diarrhoeal diseases in 1970 was 10,816 (Erwa, 1975). In 1973, children in Brazil under one year of age totalled less than one fifth of the population but suffered almost four-fifths of all deaths, while in the United States of America this age group represented 8.8 % of the population and suffered only 4.3 % of deaths (Berg, 1973). There is no doubt that improving the sanitation within a community should lead to an improvement in health, but it is difficult to ascertain whether the impact would be direct or indirect. Often, provision of better sanitation is part of broader development activities within the community, which are also including health and hygiene education (Blum and Feachem, 1983).

Man and animals are true reservoirs of salmonellae in nature, and persons with salmonellosis or convalescent or chronic carriers, are the main source of infection. Serving as the ultimate source of infection, infected individuals can excrete millions of salmonellosis bacilli in the faeces, which are the usual source of contamination of food or drink. Three per cent of cases of typhoid and paratyphoid fever become carriers and the rate increases with age and the prevalence of gall bladder disease (Manson-Bahr *et a.l*, 1987). As many as three per cent of all recognized cases of typhoid and paratyphoid fever continue to excrete *S. typhi*, *S. paratyphi* and *S. typhimurium* in faeces or urine for years following recovery. Temporary carriers, who excrete Salmonella for one to four months following infection, are also of considerable importance in disseminating the disease. Flies or other insects can carry organisms from faeces or other infected material to food or drink and have been implicated in some outbreaks. The fact that salmonellae survive freezing or drying enhances the possibility of spread by contaminated ice, dust, food, and sewage.

Outbreaks develop when defects in sanitation occur such as failure of adequate water chlorination or during natural disasters such as flood. Widespread epidemics of salmonellosis involving whole communities have been caused by contamination of drinking water with sewage, whereas lesser outbreaks usually arise from the eating of sea food contaminated with sewage or by drinking milk containing typhoid bacilli. The sex distribution of patients with typhoid fever shows no significant predilection. In recent years about 75 % of cases have occurred in persons less than 30 years of age. It seems that there is no seasonal variation in incidence of typhoid fever in areas of the world where the disease is endemic. However, (Guerrant, 1987) reported that the incidence increases in summer months.

2.2.3 Transmission

Infection of man with Salmonella species is usually the result of their transmission from sick human beings, animals, or healthy carriers to susceptible individuals. (Burrnett, *et al* 1962). *S. typhi* and *S. paratyphi*, the cause of typhoid and paratyphoid fever, affect only man and the source of infection is usually infected human being excreting bacilli in faeces, urine and from blood (Manson-Bahr *et al.*, 1987). The majority of human infections are thought to result from ingestion of focally contaminated food (food-borne disease) or water. Uncooked or raw products of animals such as eggs, milk, meat, fish and poultry have been implicated as common sources of human salmonellosis (Jay, 2000). Transmission of most excreta-related diseases is the same as those for water-related diseases, being dependent on faecal-oral transmission (water-borne and water-washed) (Francys *et al.*, 1992).

2.2.4 Pathogenesis

The salmonellosis bacilli reach the small intestine shortly after ingestion and may multiply there. The organisms may then penetrate the mucosa with minimal epithelial destruction and enter intestinal lymphatics via Peyer's patches to be carried to the blood stream. This initial early bacteraemia apparently occurs within 24 to 72 hours after ingestion of organisms and is rarely detected in natural infections because patients are usually asymptomatic at this early stage. Viable bacilli are disseminated by blood throughout the body and apparently persist within reticuloendothelial cells. Intracellular multiplication takes place and organisms re-enter the blood stream producing continuous bacteraemia that persist for some days. Recovery is unrelated to the level of titre of agglutinins against the somatic, flagellar, or Vi antigens of the bacterium.

The number of cells ingested is an important determinant for salmonellosis to resulting from exposure to salmonellae. However some studies have also demonstrated that different strains of salmonellosis bacilli vary considerably in their capacity to produce disease in humans (Guerrant, 1987). The normal flora of the upper intestinal tract is an important protective mechanism against invasion by salmonellae. It is assumed that endotoxin plays an important role in the pathogenesis and the signs and symptoms of salmonellosis. These endotoxins through response suggested that endogenous pyrogen through released by local inflammatory to salmonellae' endotoxin may sustain the pyrexia in typhoid fever (Guerrant, 1987; Ochei and Kolhatkar, 2000).

2.2.5 Clinical features and pathogenicity

The average of the incubation period is about 10 days, but may vary between extremes of 3 to 60 days depending on the infective dose. The clinical manifestation and duration of illness vary markedly from one patient to another (Guerrant, 1987).

Symptoms and signs are characterized by fever, abdominal pain and diarrhoea, which may last after only a week in a typical patient not treated with antimicrobial agents. The onset is gradual but may be sudden especially in paratyphoid with shivering and rigor. Headache is a common early symptom accompanied by malaise, anorexia, pains in the limbs and insomnia. Epistaxis is common but less so in paratyphoid. Abdominal pain is diffuse and the normal bowel function is disturbed with either diarrhoea or constipation. The spleen is enlarged in 20 % - 70 % of cases at some stage in the illness, sometimes as early as at the second or third day. Splenomegaly is of little use as a sign in diagnosis in holoendemic malarious areas where most young people have an enlarged spleen. Rose spots are hardly ever seen in dark-skined people. Liver involvement with jaundice and cholestatic changes is not uncommon (Manson-Bahr *et al.*, 1987). Usually there is leucopenia. The atypical disease lasts about 4 weeks; the onset is insidious with headache, malaise, anorexia and fever. Headache may be the first manifestation of disease and is usually generalized and severe. The fever is remittent frequently increasing in a stepwise manner from day to day as the illness develops. Abdominal discomfort, bloating and constipation are common during the early phase of illness. The temperature gradually increases for 5 to 7 days and then plateaus as a continuous or mildly remittent fever in the range of 39 to 40 ºC. The characteristic rash (rose-spot) is often observed during the second week of the disease and the lesions are

as small as 4 mm. The liver and spleen are frequently enlarged and palpable after the first week of illness (Guerrant, 1987; MacSween and Whaley,1992)

Faecal excretion of the organism usually persists for several days or weeks beyond the acute phase of illness, especially in case of enterocolitis. Misuse of antibiotics is one of the factors that may increase the duration of excretion of the organism (Buchwald and Blaser, 1984).

The complications that may take place are intestinal haemorrhage and perforation, meningitis, chondritis, periostitis, arthritis (Guerrant, 1987).
In Hong Kong it is often associated with glucose-6-phosphate dehydrogenase deficiency, and haemolytic anaemia. Severe haemolytic anaemia occurred in 2% of cases, but the cause is unknown (Manson-Bahr *et al.*, 1987).

2.2.6 Laboratory diagnostic features

Leucopenia of 3000 to 4000 cells per cubic millimeter is characteristic of the febrile phase of typhoid fever. A sudden increase in leucocyte count to 10000 cells per cubic millimeter or higher should suggest the possibility of intestinal perforation. Leucocytosis in faeces is common from the second week of disease. Urine is usually normal except for transient albuminuria during the febrile period. A tentative diagnosis of typhoid fever is made by blood culture. Organisms can be recovered by culture of blood in 70 to 90 % of patients during the first week of the disease and 30 to 40 % of patients during the third week. Blood cultures are frequently positive during relapse. Only about 10 to 15 % of patients have positive stool culture during the first week reaching the maximum of about 75 % during the third or fourth week of illness and then decline afterwards. The majority of patients, but certainly

not all, develop a four fold or greater rise in serum agglutinins against the somatic (O) antigens of the typhoid bacillus during the course of the disease. A four fold or greater increase in serum titre in the absence of recent typhoid immunization is compatible with infection with *S. typhi*. All group D organisms such as *S. typhi*, as well as organisms in groups A and B, have certain common antigens which can evoke the formation of antibodies reactive with the O antigen used in the Widal test (Guerrant, 1987). Agglutinins against flagellar (H) antigen frequently appear in higher titre than agglutinins against the O antigens. However, the H agglutinins are even more subject to nonspecific variation than O agglutinins and are of no value in diagnosis. Agglutinins begin to appear after about 1 week of illness and reach a peak titre during the fifth or sixth week. Early antimicrobial therapy may dampen the immunologic response in patient with typhoid fever (Guerrant, 1987).

2.2.6.1 Sensitivity and specificity of Widal test

As for every laboratory test or diagnostic procedure, there is a set of fundamental questions that should be asked. More important are: firstly, if the disease is present, what is the probability that the test result will be positive? This refers to the sensitivity of the test. Secondly, if the disease is absent, what is the probability that the result will be negative? This question refers to the specificity of the test. Widal test is a demonstration of Salmonella antibodies against its O-somatic and H-flagellar antigens in blood. It is used to indicate the presence of typhoid fever. However, the test is not specific, since some other Salmonella spp. (e.g. *S. enteritidis* and *S. typhimurium*) and even some other genera of bacteria and parasites such as malaria parasites can cross-react with *S. typhi* (Onuigbo, 1990; Mbuh *et al*.,

2004). So, the test is of limited value in typhoid, in which diagnosis remains essentially clinical, confirmed wherever possible by bacterial culture.

2.2.7 Treatment

The patient can be treated with good nursing care. Care should be taken with the disposal of urine and faeces. The diet should be light and nutritious and the fluid and electrolyte balance paid attention. Antibiotic treatment may have to be started before a definite diagnosis is available, on suspicion only, especially if the patient is ill (Manson-Bahr *et al.,* 1987). Below are some notes on some antibiotics used to treat salmonellosis.

Chloramphenicol

Chloramphenicol is the drug of choice and is more effective than ampicillin and co-trimoxazole in reducing the duration of fever and symptoms (Manson-Bahr *et al.*, 1987). It is most effective when given orally. Since it is bacteriostatic and not bactericidal early treatment may not lead to eradication since the immune defenses of the body have not developed and relapse may occur. It must be given in adequate doses for a period of time. A dose of 8 mg/kg four-hourly for 5 days may be adequate in patients who are not severely ill, but if fever persists at the end of 5 days, then the drug must be continued for another 5 days (Manson-Bahr *et al.*, 1987). *Salmonella* may occasionally be resistant to chloramphenicol (Pillay *et al.,* 1975). An epidemic infection has occurred in Mexico, in which the organism was chloramphenicol resistant (Pillay *et al*, 1975). Chloramphenicol, also has the disadvantage that it might induce bone marrow aplasia and it is reputed to be the leading single cause of drug-induced plastic anaemia, often on the basis of individual susceptibility.

Ampicillin

Ampicillin has been used in a doses of up to 2000 mg/day, but the failure rate and relapse is high (Manson-Bahr *et al.*, 1987). Ampicillin in a dosage of 1g six-hourly is safer than chloramphenicol but not quite as efficacious.

Amoxicillin

Compares well with chloramphenicol and is an effective alternative for the treatment of typhoid in a dose of 1 g, six-hourly for 14 days, while 2g a day for 21 days gave better results than chloramphenicol, and with no carriers and only 2% relapse rate (Manson-Bahr *et al.*, 1987). Amoxicillin, however, is better absorbed and produces serum concentrations considerably higher than those of ampicillin. There is a report of success in treating four cases of typhoid fever with Amoxicillin (Pillay *et al.*, 1975).

Co-trimoxazole (Septrin)

A dose of two 480mg tablets twice a day for 7 days has proven successful production of a more rapid clinical improvement, but a slower defervescence than chloramphenicol (Manson Bahr *et al.*, 1987).

Ciprofloxacin

This synthetic compound has antibacterial activity directed mainly against Gram negative organisms. The action is specifically against the synthesis of DNA (Hugo, 1977). Ciprofloxacin appears to be the most effective drug for the treatment of Salmonella infections (Crump, 2003). Out of over 2500 Salmonella strains tested for resistance to ciprofloxacin, only less than 1.0 % were resistant (Anon, 2003).

2.2.8 Prevention and control

Salmonellosis is one of the diseases which could considerably spread due to poor hygienic food and lack of proper sanitary measures. So, practice of hygienic measures, good sanitation and health education are important tools to prevent the disease. "Sanitation" refers to all conditions that affect health, especially with regard to dirt and infection and specifically to drainage and disposal of sewage and refuse from houses (the concise Oxford dictionary). At its first meeting in 1950, the WHO Expert Committee on Environmental Sanitation defined environmental sanitation as including the control of community water supplies, excreta and waste water disposal, refuse disposal, vectors of disease conditions, food supplies and handling, atmospheric conditions, and the safety of the working environment. The world's need for basic sanitation services (i.e. drinking-water supply, excreta and waste water disposal) have greatly increased as a result of rapid population growth and higher expectations. This led to the designation of this important issue by the United Nations of the International Drinking Water Supply and Sanitation Decade (1981-1990).

Antimicrobial treatment of typhoid fever and of asymptomatic Salmonella carriers has become increasingly complicated by the emergence of multidrug-resistant strains of *S. typhi*. However, treatment of Salmonella infections is effective, especially if ciprofloxacin and cefotaxime are used (Crump, 2003;Anon, 2003).

Vaccines against Salmonella infections in humans are less developed than in animals. Vaccination of high-risk populations is considered the most promising strategy for the control of typhoid fever. The old, heat-inactivated whole-cell vaccine showed protective efficacy rates that, in controlled studies, ranged between 51% and 67%, but this vaccine is associated with

frequent adverse reactions (WHO, 2003b). For this reason it has been replaced by newer typhoid vaccines in industrialized countries. Two currently-licensed typhoid vaccines confer protective efficacy rates comparable to those of the whole-cell vaccine, without significant side-effects. One is a parenteral vaccine based on the purified Vi polysaccharide of *S. typhi*. The other, Ty21a, is a live, attenuated vaccine that is administered orally (WHO, 2003b). Following administration according to their respective schedules, both vaccines induce protective immunity for several years. Although well-controlled effectiveness trials in this field are relatively scarce, such studies in schoolchildren suggest that large-scale vaccination of selected groups against typhoid fever may be a significant step towards control of this disease.

3. MATERIALS AND METHODS

3.1 Media used for isolation, identification and sensitivity testing

All media used in this study were obtained from Oxoid, London, unless otherwise specified.

Deoxycholate Citrate Agar

It is a selective and differential medium used to isolate Salmonella and *Shigella* species.

Formula (per litre)

Lab-lemco Powder	5.00 g
Peptone	5.00 g
Lactose	10.00 g
Sodium	8.50 g
Sodium thiosulphate	5.40 g
Ferric citrate	1.00 g
Sodium deoxycholate	5.00 g
Neutral red	0.02 g
Agar	12.00 g

Preparation: The medium was used at a concentration of 5.20 g in every 100 ml of distilled water. It was mixed and dissolved by boiling; then cooled to 50-60 °C for pH adjustment (7.2 ± 0.2). The preparation was autoclaved at 121 °C for 15 min, cooled to 45–50 °C and then distributed into Petri dishes, ~ 20 ml each, under aseptic conditions.

Selenite F. Broth Modified

Selenite F (faeces) Broth is used as an enrichment medium for the isolation of *Salmonella* species from faecal specimens and *Salmonella typhi* from urine.

Formula

Sodium hydrogen selenite	0.80 g
Peptone	1.00 g
Mannitol	0.80 g
Di-sodium hydrogen phosphate anhydrous	2.00 g
Distilled water	200 ml

Preparation: The medium was prepared by dissolving the dry ingredients in 200 ml of distilled water by heating at 75–80 °C. The mixed medium was distributed into screw-capped universal bottles, 5 ml each. Then it was sterilized by steaming for maximum 20 min.

Cary-Blair Transport Medium

Cary-Blair semi-solid transport medium is used to preserve the viability of enteric pathogens in faecal specimens.

Formula

Sodium thioglycollate	0.75 g
Di-sodium hydrogen phosphate	0.55 g
Sodium chloride	2.50 g
Agar	2.50 g

Calcium chloride 10 g/1	4.50 ml
Distilled water	495 ml

Preparation: It is prepared freshly. The dry ingredients were dissolved by boiling and allowed to cool to 50 °C before addition of 4.5 ml of the freshly prepared calcium chloride solution. The mixture was well mixed and the pH was adjusted to 8.4. Then it was distributed in 7 ml amounts in screw-capped bottles and sterilized by tyndallizition for 15 min.

Xylose Lysine Deoxycholate (XLD) Agar

It is used to isolate Salmonella and Shigella species from faecal specimens. Based on xylose fermentation, lysine decarboxylation and hydrogen sulphide produced, it is possible to differentiate Salmonella and Shigella from most non-pathogenic enterobacteria.

Formula

Yeast extract powder	3.00 g
L-lysine HCl	5.00 g
Xylose	3.75 g
Lactose	7.50 g
Sucrose	7.50 g
Sodium deoxycholate	1.00 g
Sodium chloride	5.00 g
Sodium thiosulphate	6.80 g
Ferric ammonium citrate	0.80 g
Phenol red	0.08 g
Agar	12.50 g

Preparation: The medium is used at a concentration of 5.3 g in every 100 ml of distilled water. It was dissolved by careful heating and cooled to about 55 °C. It was then dispensed aseptically in sterile Petri dishes.

Salmonella- Shigella Agar (SSA)

SSA is a selective medium used to isolate Salmonella and Shigella species from faecal specimens.

Formula

Lab-lemco powder	5.00 g
Peptone	5.00 g
Bile salts	5.50 g
Lactose	10.00 g
Sodium citrate	10.00 g
Sodium thiosulphate	8.50 g
Ferric citrate	1.00 g
Brilliant green	0.33 mg
Neutral red	0.025 g
Agar	12.00 g

Preparation: The medium is used at a concentration of 5.7 g in every 100 ml of distilled water.

MacConkey Agar

MacConkey Agar is a differential and low selective medium used to distinguish lactose fermenting from non-lactose fermenting bacteria and to select enterobacteria due to its bile salt contain.

Formula

Peptone	20.00 g
Lactose	10.00 g
Bile salts	1.50 g
Sodium chloride	5.00 g
Neutral red	0.075 g
Agar	15.00 g

Preparation: The medium is used at a concentration of 5.2 g in every 100 ml of distilled water. It was mixed and dissolved by boiling; then cooled to 50-60 °C for pH adjustment (7.2 ± 0.2). The preparation was autoclaved at 121 °C for 15 min, cooled to 45–50 °C and then distributed into Petri dishes, ~ 20 ml each, under aseptic conditions.

Kligler Iron Agar (KIA)

KIA is a differential slope medium used to assist in the identification of Salmonella, Shigella and some other enterobacteria. KIA reactions depend on the fermentation of lactose and glucose (dextrose) and production of hydrogen sulphide.

Formula

Lab-lemco powder	3.00 g
Yeast extract	3.00 g
Peptone	20.00 g
Sodium chloride	5.00 g
Lactose	10.00 g
Dextrose (glucose)	1.00 g

Ferric citrate	3.00 g
Sodium thiosulphate	3.00 g
Phenol red	0.50 g
Agar	12.00 g

Preparation: The medium is used at a concentration of 6.0 g in every 100 ml of distilled water. It was mixed and dissolved by boiling; then cooled to 50-60 °C for pH adjustment (7.2 ± 0.2). The preparation was dispensed in 6 ml amounts in large size tubes (16 x 160 mm) and autoclaved at 121 °C for 15 min and allowed to solidify in a sloped position.

Nutrient Agar and Nutrient Broth

Nutrient agar (NA) is a basic culture medium used in the preparation of blood agar and other media. It is also used as a slope medium to subculture pathogens isolated on carbohydrate-containing media prior to performing biochemical and serological tests. In semi-solid form and as an agar deep, NA is used to maintain cultures of control organisms.

Nutrient broth is used to support the growth of microorganisms that have no special nutritional requirements. Like NA, it is also used in the preparation of more enriched media.

Formula

Yeast extract	2.0 g
Lab-lemco powder	1.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar (in NA)	15.0 g

Preparation: Nutrient agar is used at a concentration of 2.8 g in every 100 ml of distilled water and nutrient broth at 1.3 g in every 100 ml of distilled water. It was dissolved by boiling; then cooled to 50-60 °C for pH adjustment (7.2 ± 0.2). The preparation was autoclaved at 121 °C for 15 min, cooled to 45–50 °C and then distributed into Petri dishes, ~ 20 ml each, under aseptic conditions. The broth medium, after pH adjustment, was dispensed in bottles or tubes in the required amounts and autoclaved as before.

Motility Indole Urea (MIU) Medium

MIU is a semi-solid medium used to differentiate between enterobacteriaceae species by their motility, urease, and indole reactions.

Formula

Tryptone	30.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.00 g
Sodium chloride	5.00 g
Agar	4.00 g
Phenol red (2.5 g/1, ethanol:water [1:1]	2.00 ml
Distilled water	1000 ml

Preparation: The medium is prepared by dissolving the dry ingredients in 1000 ml distilled water by boiling. Then it was allowed to cool 50-60 °C before addition of the indicator phenol red solution and pH adjustment (7.1±0.2). The medium is then mixed well and dispensed in 95ml volumes in screw-capped bottles and autoclaved at 121 °C for 15 min.

3.2 Widal test

A suitable pipette was used to apply each serum sample onto a clear transparent glass slide. The following amounts of serum were tested: 0.8 ml, 0.4 ml, 0.2 ml, 0.1 ml, 0.05 ml. The antigen (BDH, London) was shaken gently to ensure a uniform suspension. One drop of antigen suspension was added just at the bottom of each quantity of serum. The serum and the antigen were mixed well using a piece of applicator glass stick proceeding from higher to lower serum diluting. Each mixture formed an area of approximately ¼ inch by 1 inch. The slide was then rotated by hand for 2-3 minutes. Agglutination was observed using good indirect light against a dark back ground. Interpretation of antibody titre was as follows: 1/40 (insignificant), 1/80 (doubtful), 1/160 (suggestive or significant) and 1/320 (significant).

3.3 Antibiotic Sensitivity Testing

Sensitivity of 20 isolates to 11 antimicrobial agents was determined by the standard disc diffusion method (Buxton and Fraser, 1977). The antimicrobial agents were commercially obtained.

The antimicrobial agents used, their abbreviations, concentrations and origin have been shown in Table II. The medium used for sensitivity testing was nutrient agar.

Table II. Antimicrobial agents used, their abbreviations, concentrations and origin

Antimicrobial agent	**Abbreviation**	**Concentration (mg/1)**	**Origin**
Ampicillin	A	8	Sudan

Chloramphenicol	C	8	USA
Gentamycin	G	4	USA
Kanamycin	K	16	USA
Streptomycin	S	16	USA
Septrin (Sulphamethoxazole + trimethoprim)	St	25	Jordan
Tetracycline	T	8	Sudan
Cephalexin	Ch	30	USA
Nalidixic acid	Nx	16	Jordan
Ciprofloxacin	Cp	1	USA
Cefotaxime	Ct	1	Jordan

3.3.1 Disk diffusion method

About a half of medium–size colony of a fresh over-night SSA culture of the test isolate was properly distributed by streaking onto NA. The antibiotic discs were applied on the plates by using sterile forceps and the plates were incubated at 37 °C for 24 h. The diameter of the zone of inhibition to each antibiotic against each test isolate was estimated and the results were written.

3.4 Collection of specimens

Fifty children with persistent and non-persistent diarrhea were randomly selected from children in Dar-assalam camp. Subjects were from both sexes, five months to five years old. Stool samples were collected from all cases, while sera samples were collected only from those who were positive for Salmonella isolation. Two to three millilitres of venous blood were

withdrawn by vacutainer tubes and sera were separated in less than two hours. Stool samples were put in sterile plastic vials and then inoculated into Cary-Blair transport medium because the location was far from the laboratory.

3.5 Culture method

In the laboratory, the stool samples were inoculated in selenite f broth and incubated at 37 °C overnight. After incubation, the broth culture was streaked onto DCA, SSA, MacConkey, KIA and XLD. The plates of different media and bottles of KIA were examined after overnight incubation. Colonies with cultural characteristics and cells with microscopic features similar to Salmonella (large, moist, round and smooth colonies of short, single Gram negative rods) were especially selected and subcultured for purification. Gram-stained smears were prepared and examined from the Salmonella-like colonies. Pure cultures of Salmonella-like colonies were differentiated from similar colonies of other such as *E. coli* and *Shigella* using standard biochemical reaction of isolates the medium used is recorded and serological techniques (Ewing, 1986).

3.5.1 Purification of Isolates

The broth cultures were subcultured on DCA, SSA. MacConkey, KIA and XLD. This was achieved by several subcultures of a typical and a well-isolated colony from the corresponding medium. The process was repeated till purification was achieved. The resulting growth was cheched for purity by Gram’s stain and examined microscopically.

3.5.2 Identification of the Isolated Bacteria

3.5.2.1 Primary Identification

Gram's staining to see the shape, arrangement and Gram's reaction were performed for primary identification. Bacterial smears were prepared by emulsifying a small inoculum of the bacterial culture in a drop of normal saline and spreading it onto a clean glass slide (15-20 mm). The smears were allowed to dry on air and then fixed by gentle heating.

3.5.2.1.1 Gram's Stain

Gram's stain was applied according to Ochei and Kolhatkar, (2000). The slides (smears), were placed on the staining rack and were flooded with crystal violet stain (base stain), for 1 minute, then the stain was washed off with distilled water. The smears were covered with Lugol's iodine (mordant) 1-2 seconds, and then rinsed in distilled water. The smears were counter stained with safranin for 1 minute rinsed in water, blotted with filter paper with allowed to air-dry. The prepared slides were examined microscopically with oil immersion lens objective. Bacteria coloured violet was labeled as Gram Positive and red-coloured bacteria were labeled Gram negative.

3.5.2.2 Secondary Identification of Isolated Bacteria

This was done by using the biochemical tests, which were performed according to Barrow and Feltham (1993). As shown in Table III.

4. RESULTS

4.1 Isolation

According to cultural, microscopic and biochemical characteristics, a total of 20 Salmonella isolates were recovered from stool samples. Nine of them were *S. Typhi*, four were *S. Paratyphi* and seven were *S. Typhimurium*. Most primary cultures of the stool samples were mixed cultures (polymicrobial). Also, most of the direct wet mounts of the stool samples showed presence of both bacteria and parasites. *E. coli* was isolated ten samples and *Shigella.* Six of the direct wet mounts were found mixed with *amoeba*, with flagellates, and two with worms (*Scaris* and *Hymenolepis nana*). Isolation frequency of the different organisms is shown in Fig. 1. If the 20 stool samples from which Salmonella was isolated are considered, four were found mixed with flagellates, two with Amoebae and one with Shigella.

4.2 Identification of Salmonella isolates

4.2.1 Cultural differentiation

On MacConkey, DCA and SS media, Salmonella species were non-lactose fermenters, with round and moist colonies of about 0.5 to 1.0 mm diameter. Cultural characteristics in KIA: *S. Paratyphi* considered if red slope, and yellow butt is seen and that H_2S not produced and gas is produced from the glucose, indicated by raise up or cracking of the medium. *S. Typhi* was considered if no gas is produced. On the other hand *S. Typhimurium* was considered if it produced deep black colour with H_2S as well as gas production. On XLD medium, colonies of *S. Typhi* and *S. Paratyphi* were red-pink, round, smooth, moist and about 1-2 mm in diameter, while colonies of *S. Typhimurium* were red-pink and black in their centres.

4.2.2 Biochemical testing

The result of biochemical examination of the 20 isolates of Salmonella was illustrated in Table III. All isolates showed the same biochemical characteristics to all biochemical tests, except H_2S and gas production in KIA.

Table III. Results of biochemical characteristics of Salmonella isolates

Characteristic	***S. typhi***	***S. paratyphi***	***S. typhimurium***
Glucose fermentation	+	+	+
Indole production	-	-	-
Manitol fermentation	+	+	+
Maltose fermentation	+	+	+
Urease production	-	-	-
Gas production in KIA	-	+	+
H_2S production	+	-	+
Sucrose fermentation	-	-	-
Motility	+	+	+

4.2.3 Serology

The 20 sera collected from the cases which are found positive for Salmonella isolation were tested by Widal test. The sera from cases found positive for isolation of *S. typhi* and *S. paratyphi* had significant antibody

titre, while those from cases found positive for *S. typhimurium* had insignificant antibody titre (Table IV and Figs. 2 and 3).

Table IV. The results of Widal test

***S*. species**	**No. of isolates**	**Antibody titre (No. of isolates)**	**Remarks**
S. typhi	9	1/80 (1) 1/160 (5) 1/320 (3)	Doubtful Significant Significant
S. paratyphi	4	1/160 (4) 1/320 (1)	Significant Significant
S. typhimurium	7	1/40 (7)	Insignificant

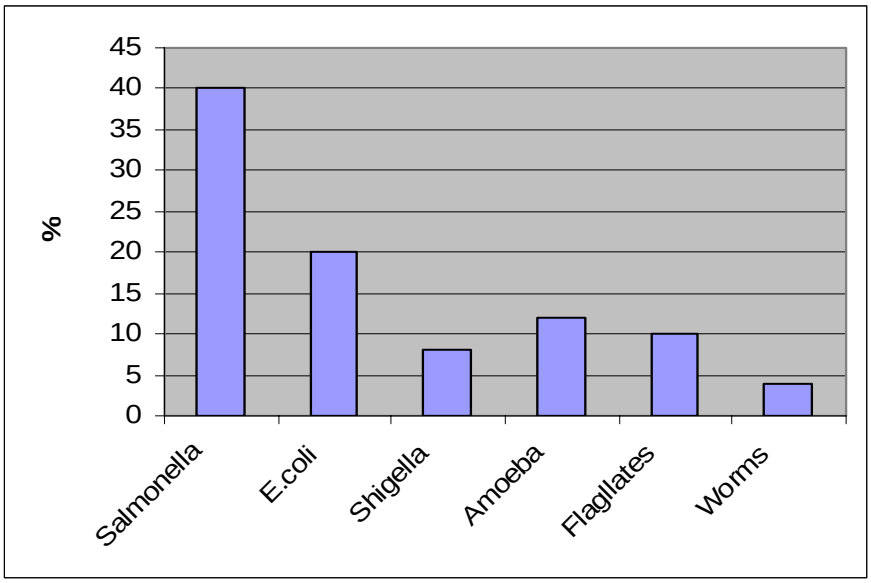

Fig. 1: Incidence of different organisms isolated from stool samples.

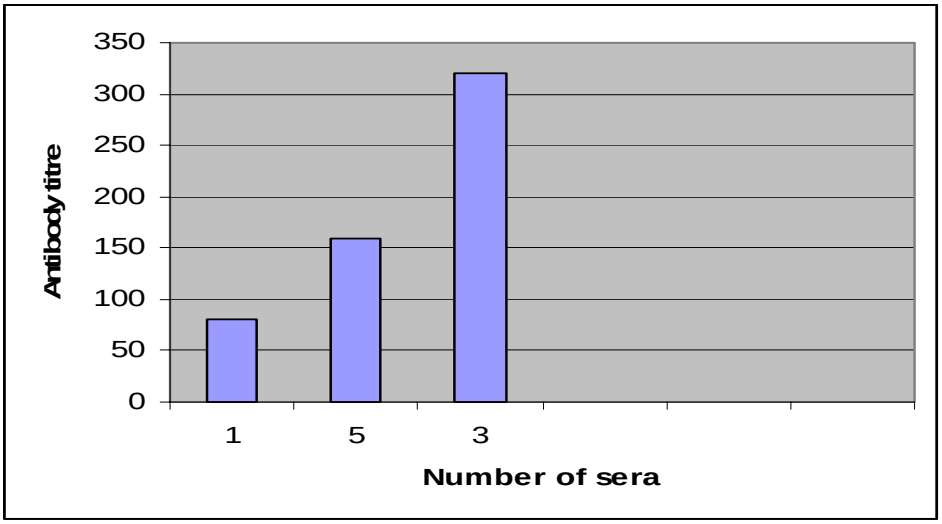

Fig. 2: Antibody titre of sera from cases positive for *S. typhi*

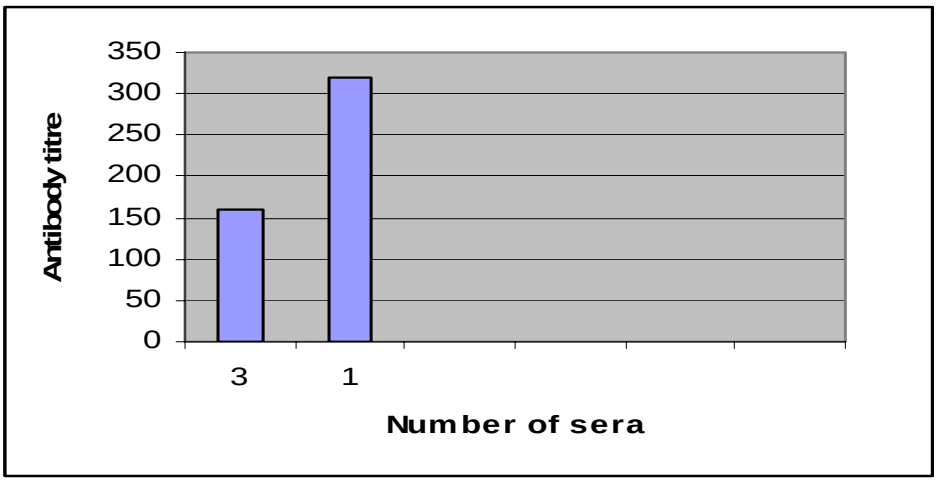

Fig. 3: Antibody titre of sera from cases positive for *S. paratyphi*

4.3 Antibiotic susceptibility

The antibiotic sensitivity test was conducted for the 20 isolates of Salmonella spp. against eleven antimicrobial agents. The antimicrobial agents used, their abbreviations, concentrations and origin were shown in Table II. The sensitivity of all test isolates to the different antimicrobial agents is shown in Table V. All the isolates of the three *Salmonella* spp. were very highly sensitive to ciprofloxacin, high sensitive to cefotaxime and moderately sensitive to Septrin. Isolates of *S. typhi* and *S. paratyphi* were moderate to high sensitive to chloramphenicol, while all isolates of *S. typhimurium* were resistant. All the isolates of the three species were resistant to ampicillin, gentamycin, kanamycin, streptomycin, tetracycline, cephalexin and nalidixic acid.

Table V. Results of antibiotic susceptibility of Salmonella isolates

S. species	A	C	G	K	S	St	T	Ch	Nx	Cp	Ct
S. typhi	R	S	R	R	R	S	R	R	R	S	S
S. paratyphi	R	S	R	R	R	S	R	R	R	S	S
S. typhimurium	R	R	R	R	R	S	R	R	R	S	S

R = either very narrow zone of inhibition or no inhibition

S = narrow to wide zone of inhibition

5. DISCUSSION

This study shows clearly that Salmonella is one of the important bacterial, which cause diarrhoea in children in the areas where displaced people. It was isolated from 20 (40 %) of stool samples. This result was in accordance with a previous report by Schelotto *et al*. (1991) that Salmonella is one of the microorganisms most frequently associated with diarrhoea in children from low-income families. However, Erwa (1966) reported that Salmonella was found next to *E. coli* and *Shigella* as a cause of diarrhoeal diseases in Sudanese children. In this study, three species were encountered, namely *S. Typhi, S. Paratyphi* and *S. Typhimurium* with prevalence of 18 %, 8 % and 14 %, respectively. The highest frequency of isolation found for *S. Typhi* is in agreement with the findings of Mohamed (1999); however, Erwa (1966) found that *S. Paratyphi* C was the most prevalent in Sudan.

Isolation results of *S. Typhi* and *S. Paratyphi* were confirmed by serology (Widal test), while sera of cases positive for S. *Typhimurium* isolation were negative (insignificant antibody titre). The Widal test was positive for *S. Typhi* and *S. Paratyphi* and not *S. Typhimurium* because it is an agglutination test using bacterial suspensions of *S. Typhi* and *S. Paratyphi* treated to retain only the somatic and/or flagellar antigens of each species. Cross-reaction between these three species as well as some other *S.* spp. is possible due to certain common antigens (Guerrant, 1987; Onuigbo, 1990; Mbuh *et al*., 2004). Co-infections in the cases studied were also detected. Most of the primary cultures of the stool samples were mixed cultures. *E. coli* was isolated from ten (20 %) of them and *Shigella* from four (8 %) of them. Also, in the direct wet mounts, *amoeba* was observed in six (12 %) of the stool samples, flagellates in five (10 %) and worms (*Scaris* and *H. nana*)

in two (4 %) of these samples. This was true for both acute and persistent cases of *diarrhoea.* Likely, such results of co-infection were previously reported by Erwa (1966) and Schelotto *et al.* (1991). This co-existence of other bacteria and parasites may partly play a role in the causation of diarrhoea in the Salmonella-positive cases. Either predispose to Salmonella infection or act together with Salmonella to produce such diarrhoea (multifactorial). Also, these organisms can act as a primary cause of similar diarrhoeal diseases (Smith, 1949; W.H.O Report, 1961; Joe *et al*., 1966). *E. coli* and *Shigella* were reported as constantly present in mixed infections causing diarrhoea in children in Sudan (W.H.O. Report, 1961), as well as similar results were also obtained in other countries (Lie *et al*., 1966). However, Erwa (1966) reported that flagellates are fairly the common pathogens in diarrhea of children in this country. There are many Salmonella spp. reported as human pathogens (Cheesbrough, 1984; W.H.O., 2003a); however, in this study, only *S. Typhi, S. Paratyphi* and *S. Typhimurium* were isolated. *S. typhi* and *S. paratyphi* affect only man in tropical countries (Cheesbrough, 1984), that is why they are commonly isolated in Sudan. However, *S. Typhimurium* is found in animals and it could be transmitted to humans and cause infection (Jawetz, 1966). *S. Typhimurium* was not found reported before as one of causes of diarrhoeal diseases in children in Sudan. Other Salmonella spp. were not isolated in this study; and this may be due to different environment or conditions which may need further research.

The antibiotic susceptibility test was performed for the 20 Salmonella isolates against eleven antimicrobial agents. All the isolates were highly sensitive to ciprofloxacin, which was found to be the most potent antibiotic used. Similarly Crump (2003) reported that Ciprofloxacin appears to be the

most effective drug for the treatment of Salmonella infections. The action of this drug is mainly directed against Gram negative bacteria (Hugo, 1977). Out of over 2500 Salmonella strains tested for resistance to ciprofloxacin, only less than 1.0 % were resistant (Anon, 2003). The next most potent antibiotic was cefotaxime. It is effective against Salmonella spp. and other Gram negative bacteria (Guerra *et al*., 2000). All *S. Typhimurium* isolates were resistant to chloramphenicol, while *S. Typhi* and *S. Paratyphi* isolates showed high to moderate sensitivity to it. Pilay *et al*. (1975) reported that chloramphenicol is the drug of choice for *S. Typhi* and *S. Paratyphi*, however, some salmonellae may naturally or occasionally be resistant to it. Resistance to chloramphenicol was reported in England and Mexico (Manson-Bahr *et al.*, 1978). Chloramphenicol has the disadvantage that it might induce bone marrow aplasia and it is reputed to be the leading single cause of drug-induced plastic anaemia. All the isolates of the three species were moderately sensitive to Septrin. Septrin has been successfully used for treatment of typhoid fever (Manson-Bahr *et al*., 1978). All the Salmonella isolates were found resistant to the other antibiotics. Manson-Bahr *et al.* (1978) reported that multidrug-resistance in S*almonella* spp. was very common.

The results of the antibiotic susceptibility showed that the isolates were sensitive only to few antibiotics and this may indicate emerging resistance to old antibiotics. Therefore, in routine laboratory diagnostic work, beside pathogen isolation and identification, control measures should also include drug sensitivity test.

Recommendations

This research was carried out in Dar-assalam camp, which is one of the remote areas in Omdurman town. The citizens of this area are displaced people who came from western and southern Sudan during the civil war. The area is lacking basic services of health care, water supplies and toilets. There are different sources of diseases in the area, therefore, my recommendations to the government, social, religious and humanitarian organizations is to protect these people from diseases such as salmonellosis by doing the following:

1. Health education to practice hygiene in their life.
2. Making sanitary toilets.
3. Provision of clean healthy water.
4. According to the study results, salmonellosis is a major problem, so, continuous testing and treatment with ciprofloxacin is a highly recommended approach.

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